PROTEIN TRAFFICKING DEFECTS IN INHERITED RENAL DISEASE

Introduction

Protein trafficking is the mechanism by which a cell transports proteins to appropriate position. Proteins must travel from polyribosomes to many different sites in the cell to perform their designated functions. Abnormal intracellular traffic of proteins and their sorting can result in inherited renal diseases. The topic is discussed under the following headings :

1. The process of intracellular protein trafficking
2. Therapeutic targets for abnormal protein trafficking
3. Inherited renal diseases associated with abnormal protein trafficking

THE PROCESS OF INTRACELLULAR PROTEIN TRAFFICKING

Proteins are synthesized either on free or on membrane-bound polyribosomes. Proteins synthesized on membrane-bound polyribosomes contain a signal peptide that mediates their attachment to the membrane of the endoplasmic reticulum(ER) (Rough endoplasmic reticulum branch). Proteins synthesized and sorted in the rough ER branch include many proteins that are destined to

- Endoplasmic reticulum
- Golgi apparatus
- Lysosomes
- Plasma membrane
- Secretion

Proteins synthesized on free polyribosomes lack this particular signal peptide and are delivered into the cytosol (cytosolic branch). There they are directed by specific signals to

- Mitochondria
- Nuclei
- Peroxisomes or
- Cytosol - if they lack a signal [1,2]

PROTEIN SORTING IN THE ROUGH ENDOPLASMIC RETICULUM BRANCH

Protein synthesized on membrane-bound polyribosomes contains a peptide extension (signal peptide). The proteins move through cellular compartments to specific destinations. There are specific sequences or compounds that direct proteins to specific organelles. (Fig.1)
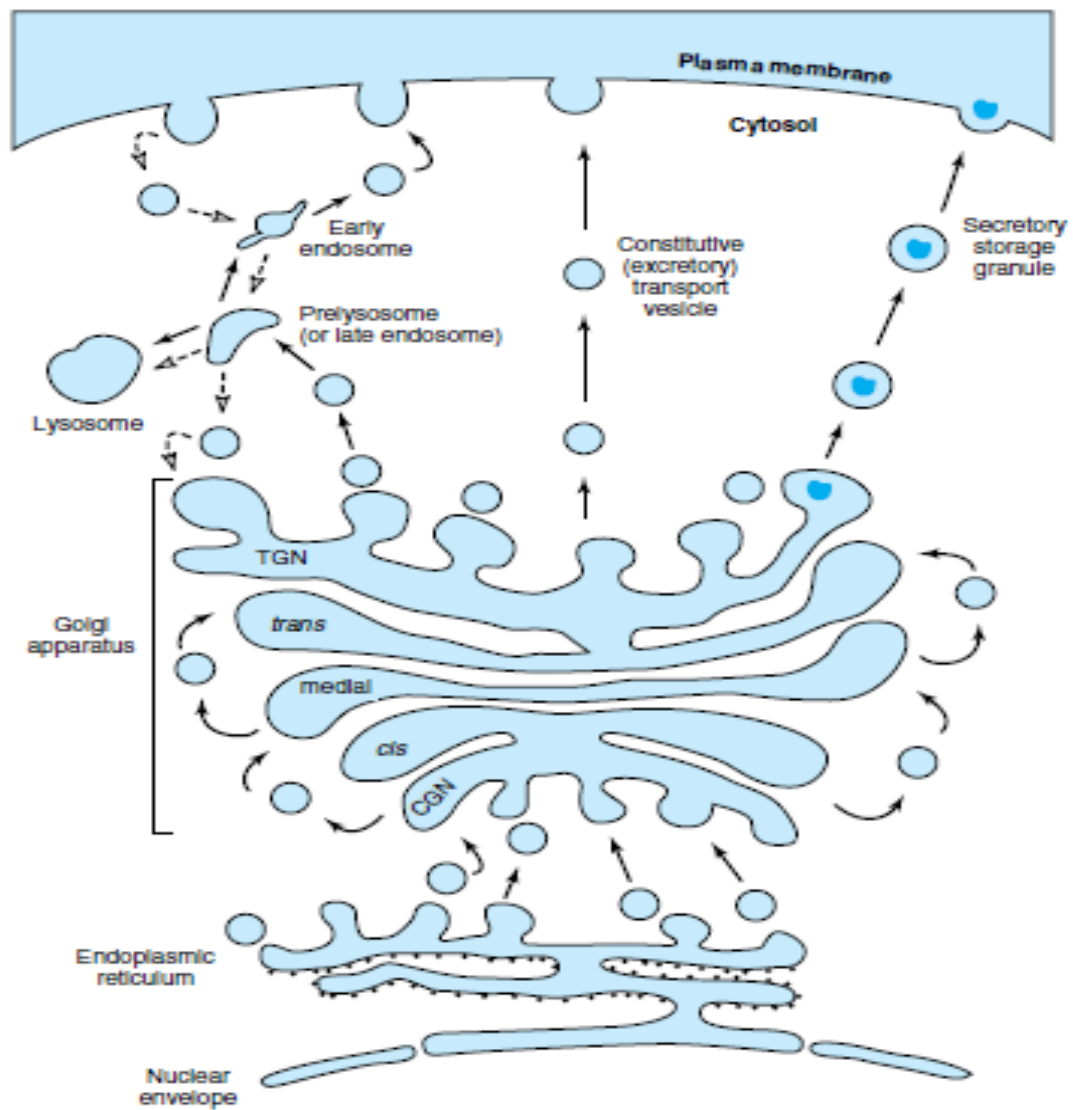
Figure 1[3]

Newly synthesized proteins are inserted into the ER membrane from membrane-bound polyribosomes. Those proteins that are transported out of the ER (solid black arrows) pass through sub compartments of the Golgi till they reach the trans Golgi network (TGN), the exit side of the Golgi. In the TGN, proteins are segregated and sorted. Secretory proteins accumulate in secretory storage granules from which they are expelled (upper right). Proteins destined for the plasma membrane or those secreted in a constitutive manner are carried to the cell surface in transport vesicles (upper middle). Some proteins may reach the cell surface via late and early endosomes. Other proteins enter prelysosomes (late endosomes) and are selectively transferred to lysosomes - the endocytic pathway (upper left).

Vesicular transport

The Golgi apparatus is Involved in glycosylation and sorting of proteins. Protein coats help recruit and concentrate their cargo, and also deforms the membrane to help budding. COP I (coating protein 1) is used for Golgi → ER. COP II (coating protein 2) is used for ER → Golgi. Exception to this rule is Golgi → endosome → lysosome, which uses clathrin. Each transport vesicle bears a unique marker consisting of one or more **v-SNARE proteins,** while each target membrane bears one or more **complementary t-SNARE proteins.** [9]

Molecular chaperones

Newly synthesized proteins may require assistance to reach their folded native-state. Certain proteins play a role in the assembly or proper folding of other proteins. Such proteins are called **molecular chaperones.** [5, 6] They stabilize unfolded or partially folded intermediates, allowing them time to fold properly, and prevent inappropriate interactions. Many are heat shock proteins (Hsp). Examples

- BiP (immunoglobulin heavy chain binding protein)
- GRP94 (glucose-regulated protein)
- Calnexin
- Calreticulin

Proteostasis network in the ER [7, 8]

Accumulation of misfolded proteins leads to activation of signaling mechanisms - include increasing its folding capacity and other responses to restore cellular homeostasis. If sustained impairment of folding occurs, cell death pathways (apoptosis) are activated. This overall process is named the **unfolded protein response (UPR).** Proteins that misfold in the ER are transported to proteosomes present in the cytosol. Chaperones present in the lumen of the ER and in the cytosol target misfolded proteins to proteosomes. Prior to entering proteasomes, most proteins are ubiquitinated and are escorted to proteasomes by polyubiquitin-binding proteins. This process is referred to **endoplasmic reticulum associated degradation (ERAD).** Figure 2.
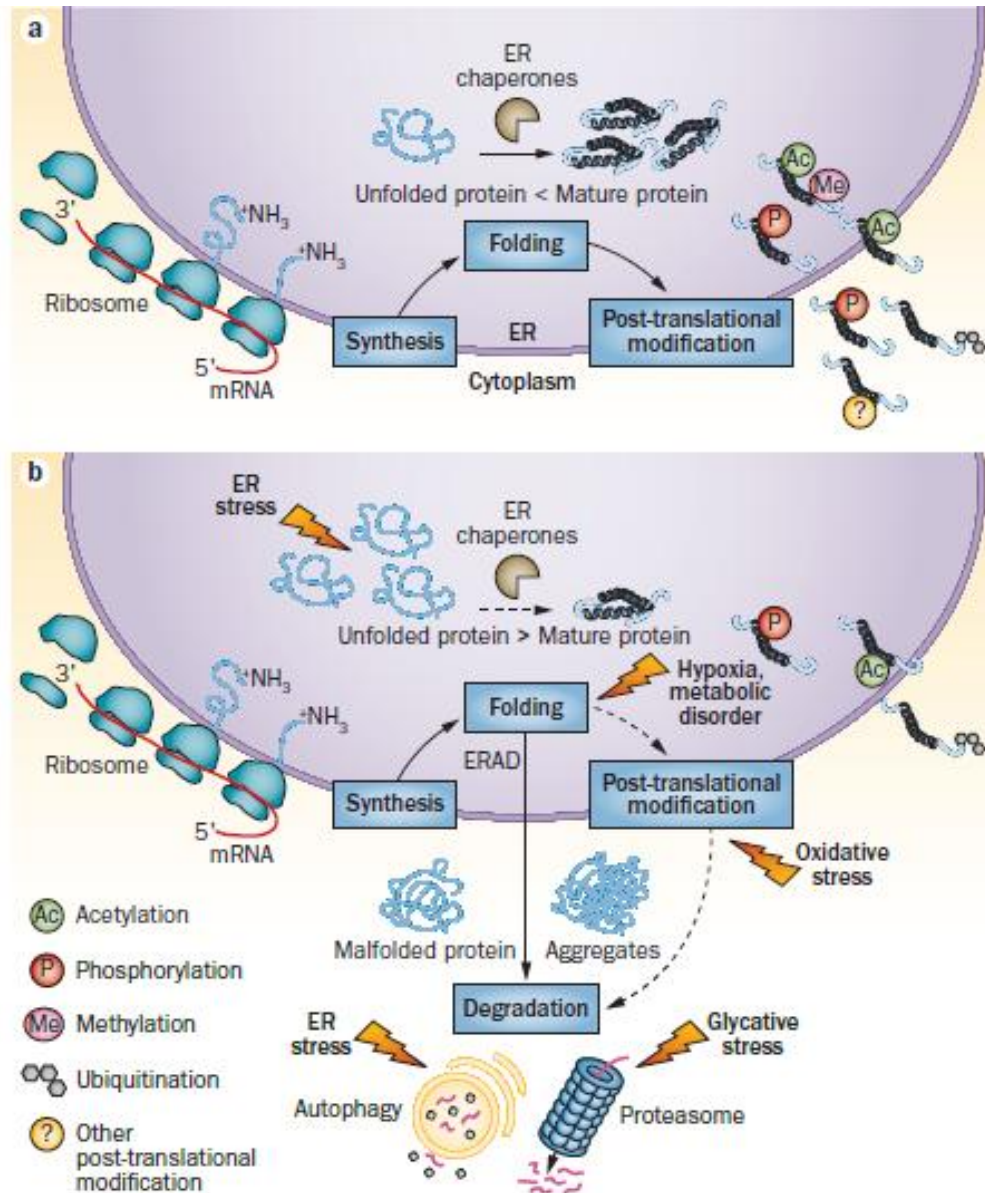
Figure 2 - Proteostasis networks in the ER. [7]

a. Under normal conditions, newly synthesized (unfolded) proteins in the ER are folded by the action of ER chaperones and post-translationally modified

b. Under conditions of stress, imbalance between synthesis and folding of proteins results in accumulation of malfolded proteins, a process termed ER stress. These abnormal proteins are degraded by the endoplasmic reticulum associated degradation (ERAD) pathway

THERAPEUTIC TARGETS FOR ABNORMAL PROTEIN TRAFFICKING

In many genetic diseases with protein trafficking defects there are mutations affecting protein folding. For these protein misfolding diseases (conformational diseases) the ideal therapeutic approach would be to identify small molecules that can bind to and stabilize the native state of mutant proteins, thereby increasing the level of folded protein and improving the protein trafficking in their original state.

Three different types of small molecules:

1. Chemical chaperones
2. Pharmacological chaperones
3. Proteostasis regulators.[12]

Chemical chaperones

Chemical chaperones are compounds with a low molecular weight that stabilize the native conformation of proteins. They do not directly bind to misfolded proteins, but stabilize them by reducing free movement and increasing hydration.

- Polyols (Glycerol and trehalose)
- Amino acids (Taurine and arginine)
- Methylamines (Betaine and trimethylamine N-oxide)

However, the therapeutic use is limited due to two reasons. First, high concentrations are needed to achieve significant increases in protein function in vivo and such concentrations are often toxic. Secondly, due to the underlying mode of action, chemical chaperones lack specificity and this may be associated with undesired effects. [11]

Pharmacological chaperones

Pharmacological chaperones are small molecules that specifically bind to a misfolded protein and can induce conformational stabilization. These molecules could either bind or stabilize the native state of the target protein or they could bind to non-native folding intermediates, serving as a scaffold for subsequent folding attempts.

Pharmacological chaperones bind with high affinity to their targets and can be effective at low concentration. Binding at or near natural ligand-binding sites would require displacement of the chaperone to rescue protein function. The variety of mutations in a particular disease may limit the use of pharmacological chaperones because they are specific. Only two pharmacological chaperones are currently approved for clinical use

1. Sapropterin dihydrochloride for phenylketonuria and
2. Tafamidis for transthyretin-related hereditary amyloidosis

Candidate molecules for pharmacological chaperones can be identified by two different strategies, hypothesis-free high throughput screenings (HTS) exploring libraries of thousands of existing chemicals and drugs, or hypothesis-driven approaches. [12]

Proteostasis regulators

Intracellular Proteostasis regulators offer a broader therapeutic approach. (Figure 3) It can be linked to four functional intracellular principles. These are

(i)   Regulation of protein production
(ii)   Chaperone upregulation
(iii) Modification of chaperone function
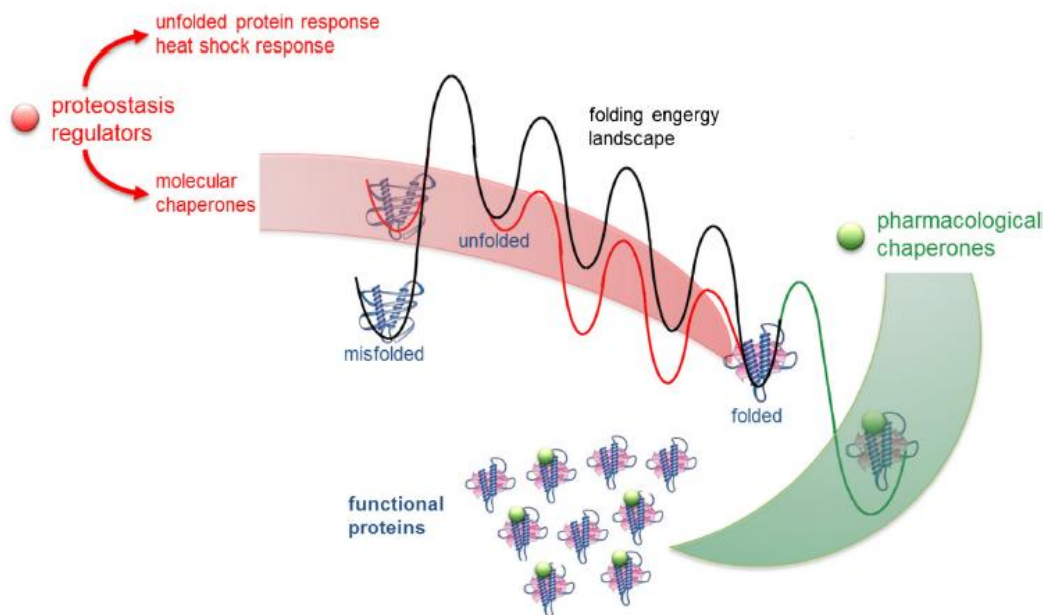(iv)   Enhanced degradation of misfolded proteins.

Figure 3

Proteostasis regulators can activate the cellular protein quality control (e.g., unfolded protein response in the ER, heat shock response in the cytosol) .Proteostasis regulators can also directly enhance the function and activity of molecular chaperones. As a result, the protein folding capacity increases and protein misfolding is reduced (red arrow). Pharmacological chaperones specifically bind to target proteins and promote their conformational stabilization (green arrow). [7]

Examples:
- Guanabenz(an antihypertensive drug) in Type I diabetes
    - modifies unfolded protein response
- 4-Phenylbutyrate - in cystic fibrosis, displays histone decatylase inhibitor activity and
    additionally exerts a chemical chaperone effect [10, 13]

INHERITED RENAL DISEASES ASSOCIATED WITH ABNORMAL PROTEIN TRAFFICKING

To date, mutations in around 120 genes are known to be responsible for monogenic diseases affecting the kidney [14]. About one-third of them directly interfere with protein transport. Different molecular mechanisms that primarily lead to defective protein transport include

1. Endoplasmic reticulum retention
2. Mistargeting
3. Defective endocytosis
4. Defective degradation

ENDOPLASMIC RETICULUM RETENTION

Endoplasmic reticulum plays a major role in protein trafficking. Retention in the ER is seen in many diseases and it is due to defective mutations affecting protein folding. (Figure 4)
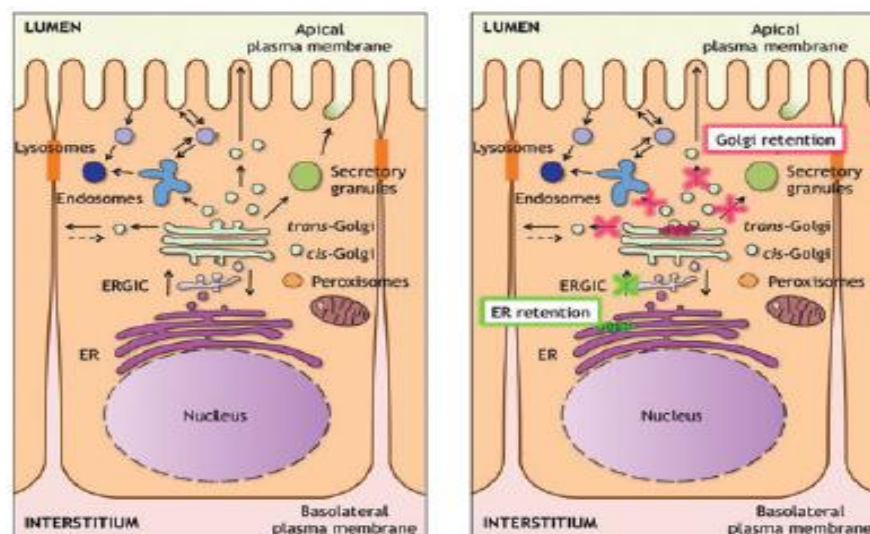

Figure 4[10]

Nephrogenic diabetes insipidus (NDI)

Arginine vasopressin binds to AVP type 2 (AVP2) on the basolateral aspect of collecting duct. AVP2 receptor stimulation causes activation of adenylate cyclase resulting in activation of protein kinase A and phosphrylation of aquaporin 2 in endosomal compartment. This leads to fusion of endosomal vesicles containing AQP2 with the apical plasma membrane where AQP2 allows water reabsorption. Once water balance is restored, AVP level decreases and AQP2 is removed from the plasma membrane by ubiquitylation-mediated endocytosis. Congenital NDI is caused by mutations in the AVPR2 or AQP2 genes [15]

X-linked form of congenital NDI

        The X-linked form of congenital NDI is caused by loss of- function mutations in the AVPR2 gene. Mutations can be divided into four classes on the basis of their molecular mechanism of action. Class 2 mutations (misses, in-frame insertion/deletion) are the most common and cause misfolding of the protein, its retention in the ER and degradation.

Therapeutics

1) In mutations which retain some functional activity the treatment approach is to induce their escape from endoplasmic reticulum and their insertion at the plasma membrane. This can be attained through

- Chemical chaperones- Glycerol or Diethyl sulfoxide
- Pharmacological chaperones-   These molecules bind to AVPR2 and aid the folding of mutated protein and its exit from the ER (SR49059)

2) To directly activate intrinsically functional AVPR2 mutants within the ER. This leads to G-protein coupled receptor activation and cyclic AMP increase, eventually resulting in increased AQP2 delivery to the plasma membrane - OPC51, VA(9990)88.

3) To bypass AVPR2 function and induce AQP2 localization at the apical membrane - agonists of E-prostanoid receptors EP2 and EP4 in rodent models. [10,16]

Autosomal forms of NDI

        Autosomal forms of NDI are due to mutations in AQP2. In autosomal dominant NDI mutations in the carboxy terminal leads to missorting signals. This leads to mislocalisation of AQP2 to golgi, lysosomes or basolateral membrane. In autosomal recessive NDI mutation leads to protein misfolding, ER retention and proteosomes degradation.

Therapeutics

        In autosomal dominant NDI, treatment is aimed at increasing cyclic AMP and hence AQP2 phosphorylation is effective. This is done with Rolipram, a phosphodiesterase 4 inhibitor, in a murine model of autosomal dominant NDI .In autosomal recessive NDI, modulating protein folding forms the treatment.[10] A Hsp90 inhibitor (17-AAG) has been tried to rescue AQP2 trafficking in a murine model.[17,18]

ER retention in inherited glomerular disorders

        In certain inherited glomerular disorders protein misfolding is proposed as a mechanism of disease.

- Nephrotic syndrome type 1 with nephrin mutations
- Alport syndrome- mutations in collagen IV
- Pierson syndrome - mutations in laminin

        Unfolded protein response plays a role in these disorders. However the effect of modulating protein folding is still unclear and needs further investigation.[10, 19, 20]

MISTARGETING

Mistargeting of the protein results in protein reaching an alternative site instead of its original site of action.

Primary hyperoxaluria type I

Mistargeting is one of the causes of primary hyperoxaluria type I (PH1). PH1 is a autosomal recessive disease caused by mutations in the AGXT gene coding for the liver-specific peroxisomal enzyme alanine-glyoxylate aminotransferase(AGT). Deficiency in the activity of AGT causes accumulation of glyoxylate and consequently oxalate. This leads to nephrolithiasis.

The mutations can have varying consequences altering protein function (loss of catalytic activity), structure (increased degradation) or subcellular localization (aberrant mitochondrial localization). The substitution of a proline with a leucine at position 11 creates a mitochondrial targeting sequence (MTS). This MTS is normally ineffective, as dimerization of the protein masks the binding motif .In some AGT mutations, the MTS becomes accessible and AGT is imported to the mitochondria instead of peroxisomes.[21]

Therapeutics

Administration of pyridoxine, a metabolic precursor of pyridoxal phosphate (PLP) has shown response in one third of patients mainly carrying G170R and F152I mutation. Increased intracellular PLP is able to act as a prosthetic group (increasing catalytic activity) and as a chemical chaperone (increasing expression and peroxisomal import).

Pharmacological chaperones to ameliorate protein folding and redirect mutant protein to peroxisomes are under investigation. [22]

Distal renal tubular acidosis (dRTA)

Inherited forms are due to mutations in proteins in the intercalated cells regulating acid base transport including anion exchanger 1 (AE1), H+-ATPase or cytosolic carbonic anhydrase II (CA II). Mutations in AE1 lead to both autosomal dominant and recessive forms of dRTA. AE1, a chloride-bicarbonate exchanger, is encoded by the SLC4A1 gene. It is expressed at the basolateral membrane of alpha-intercalated cells and at the plasma membrane of erythrocytes. Mutations may lead to ER retention and degradation or mistargeting of mutant protein to apical instead of basolateral membrane.[23, 24] Most of the dRTA-associated dominant mutations do not alter the trafficking and function of AE1 in erythrocytes. Erythrocytes probably have specific chaperones. One such molecule could be glycophorin A (GPA), an erythroid specific AE1-binding protein.

Mutations in the H+-ATPase and CA II are responsible for recessive forms of dRTA.[25]Mutants are partly trafficked to the basolateral membrane (e.g. S773P), or retained in the Golgi compartment (e.g.G701D) or in the ER (S667F).[10,25]

Therapeutics

In ER-retained dominant mutants treatment has been tried through inhibition of their interaction with the ER chaperone calnexin (by treatment with castanospermine) or with Hsc70 (by treatment with MAL3). Two small molecules (C3 and C4) that had shown increase folding efficiency in cystic fibrosis are partially successful. Such treatments were not effective on the Golgi-retained mutant. [10]

DEFECTIVE ENDOCYTOSIS

Endocytosis is a fundamental cellular process that involves internalization of extracellular molecules, plasma membrane proteins and lipids. The principal components of the mammalian endocytic pathways are early and late endosomes and lysosomes.

Dent's disease

Dent's disease is an X-linked recessive disorder that is characterized by dysfunction of the proximal tubule renal Fanconi syndrome. The disease is due to defective endocytosis in the proximal tubule. The disease is genetically heterogeneous caused by mutations in the CLCN5 gene in 60% of patients (Dent's disease 1), OCRL1 gene, also responsible for Lowe syndrome, in 15% of patients (Dent's disease2) or unidentified mutations in the remaining 25% of patients.

CLCN5 encodes the chloride/proton (2Cl−/H+) exchanger ClC-5. In the kidney, ClC-5 is expressed in the proximal tubule and also in TAL and in the α-intercalated cells of the collecting duct. In the proximal tubule, ClC-5 is mainly found in subapical endosomes and also in apical plasma membrane. ClC-5 activity contributes to endosomal acidification. The mechanism by which ClC-5 mutation leads to vesicular trafficking defect is still unknown. It is possibly due to loss of key interactions of ClC-5 with components of the intracellular trafficking machinery such as cofilin, an actin-depolymerizing protein, the Na+-H+ exchange regulatory factor-2 and Nedd-4.

Class 1 mutations lead to ER retention followed by proteasomal degradation. Class 2 mutant proteins are only partially retained in the ER. There is delayed protein maturation, reduced protein stability and increased degradation. Class 3 mutations do not affect protein folding and stability. They have only reduced or no ion permeation.

Therapeutics

Ideally molecules restoring folding and stability of mutant ClC-5 should have been effective in rescuing Class 1 and 2 mutants. However the use of sodium butyrate (PBA) or low temperature conditions which were successful in cystic fibrosis were not successful in Dent's disease. This could be due to severe protein misfolding. Newer pharmacological chaperones are being tried.[26]

DEFECTIVE DEGRADATION

Defective vesicular trafficking and degradation of proteins can modify their amount at the plasma membrane. Defect in ubiquitination results in defective endocytosis and degradation.

Diseases associated are

- Liddle syndrome
- Pseudohypoaldosteronism type 2 (PHAII).

Liddle syndrome

Liddle syndrome is a autosomal dominant disease characterized by early onset salt-sensitive hypertension. The disease is due to mutations in SCNN1B and SCNN1G genes coding for the β and γ subunit of the epithelial sodium channel (ENaC), respectively .In the kidney, it is found at the apical membrane of collecting duct principal cells.   Mutations are mainly located in the C-terminal region and lead mutation in a proline or tyrosine residue. (Pro-Pro-x-Tyr). This motif acts as the binding site for NEDD4-2, an E3 ubiquitin ligase. The WW domains of NEDD4-2 interact with the ENaC PY motif to induce its ubiquitylation. Defect in ubiquitination results in defective endocytosis and degradation. Mutations hence exert a gain- of-function effect by inhibiting the binding of NEDD4-2 and consequently leading to an increased amount of active channel at the apical membrane. [27]

Therapeutics

Specific blockers to prevent ENaC over activation or use of specific protease inhibitors are the possible therapeutic options which are being explored. [27]

Pseudohypoaldosteronism type 2

PHAII is due to increased activity of Na^+Cl^- co-transporter (NCC) at the apical membrane of the distal convoluted tubule leading to hypertension, reduced distal Na^+ delivery and consequent hyperkalaemia. Mutations were found in the two main regulators of NCC, the with-no-lysine kinases WNK1 and WNK4. Recently, mutations in the genes encoding the Kelch-like protein 3 (KLHL3)/Cullin 3 (CUL3) ubiquitin protein–ligase complex have been identified in PHAII patients. Such mutations result in defective NCC ubiquitylation and degradation. Ablation of Nedd4L in the mouse resulted in a phenotype resembling PHAII. This suggests that NEDD4-2 also plays a role in the regulation of NCC activity.

Targeting NEDD4-2 activity is a possible strategy to treat hypertension in these diseases. [28]

Pharmacological chaperone compounds for the treatment of lysosomal diseases

Fabry disease is a genetic disorder due to deficiency of the lysosomal enzyme α-galactosidase causing the accumulation of globotriaosylceramide in several tissues.

Migalastat hydrochloride is an imino sugar that selectively binds and stabilizes α-Gal A which leads to enhanced cellular levels and activity. Some studies pointed toward an elevated α- galactosidase A activity in blood, skin, and kidney with good tolerability with Migalastat hydrochloride. However, phase 3 studies failed to demonstrate the benefit of migalastat hydrochloride over placebo with statistical significance. Newer pharmacological chaperones for the treatment of Fabry disease are in various stages of clinical trials. [29]

Conclusion

Recent advances in protein trafficking and the defects associated with its disturbances have improved our understanding about many inherited renal diseases. The understanding of changes in cellular milieu and function will certainly pave way for newer therapeutic options.

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